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Permethrin

Assessment of Chronic and Oncogenic Effects

A Summary

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Permethrin

Assessment of Chronic and Oncogenic Effects

I. Availability and Usefulness of Studies:

Seven long-term chronic feeding/oncogenicity studies have been submitted to EPA in support of requests to register food and other uses of Permethrin.

Table I

Studies Submitted and Reviewed

Study	Animals (No/Sex/Group)		Duration (Weeks)	Dosage Levels (Nominal ppm)
A. Mouse:				
1. ICI	70		98	0, 250, 1000, 2500
2. FMC-I	75		104	0, 20, 500, 4000
3. FMC-II	75	M	104	0, 20, 500, 2000
		F		0, 20, 2500, 5000
4. B-W	100		92	0
	75			10, 50, 250*
B. Rat:				
1. ICI	60		104	0, 500, 1000, 2500
2. FMC	60		104	0, 20, 100, 500
3. B-W	60		104	0, 10, 50, 250*

* mg/kg/day   M = Male; F = Female

The test material for all studies, with the exception of the B-W studies, was a 40:60 cis:trans isomer ratio of Permethrin. For the B-W studies, the cis:trans isomer ratio was 25:75. Although the cis isomer is known to be more toxic than the trans isomer (based on the results of acute oral and other toxicity studies), the difference in isomer ratios used in the chronic studies was not considered substantial enough to warrant separate consideration of the B-W studies.

One study, the FMC Mouse I study, was flawed by dose level changes in the mid and high level groups and more seriously by an animal identification problem. For these reasons the study was judged to be of no usefulness in evaluation of Permethrin toxicity or oncogenicity.

One other study, the FMC Rat study, has been judged to be of very limited usefulness with regard to evaluation of lung tissues for tumors. This resulted from a failure to treat lungs from control and test animals in a comparable manner during the preparation of these tissues for microscopic examination.

The remaining five chronic studies have been determined to be useful for the purpose of evaluating the oncogenic effects of Permethrin. Four of these, the exception being the FMC Mouse II study, were also useful in evaluation of the chronic toxicity.

II. Synopses of Individual Long-Term Studies:

ICI Mouse Study - Central Toxicology Laboratory, ICI Report No. CTL/P/358 and CTL/P/359 January 27, 1978

Doses of 0 (control), 250, 1000 and 2500 ppm of Permethrin were administered in the diet to 70 male and 70 female Alderly Park strain mice/group for 98 weeks. Male and female mice were sacrificed at 26 and 52 weeks (total 141) and the remainder at 98 weeks. Relevant non-oncogenic effects observed during the study were increased mortality, increased liver enzyme (aminopyrine-N-demethylase) activity, increased liver weights, and eosinophilia of hepatocytes in both males and females at 2500 ppm. Liver changes observed in this study were considered to be related in large measure to the induction of liver microsomal enzyme activity. Minimal liver changes were also observed at 1000 ppm, but not at 250 ppm.

Table II

ICI Mouse Study

Number of Animals with Lung Neoplasms

Type	Males (ppm)				Females (ppm)			
	0	250	1000	2500	0	250	1000	2500
Adenoma	11/70 *(15.7)	6/70 (8.6)	13/70 (18.6)	17/70 (24.3)	11/70 (15.7)	8/70 (11.4)	10/70 (14.3)	15/70 (21.4)
Adenocar-cinoma & Adenoma	0	0	0	0	0	1	1	1

* % incidence

As indicated in Table II, a slight increase in lung adenomas was observed in male mice in this study. Statistical analysis of this finding revealed a positive trend for this lesion in the treated males (Armitage test was significant at p of about 0.01). Pair-wise comparisons using Fisher's Exact Test are presented below for male adenomas and for female adenomas/adenocarcinomas.

Fisher's Exact Test Results

	Males	Females
Control vs High	11/70 vs 17/70 p = 0.145	11/70 vs 16/70 p = 0.197*
Control vs Low	11/70 vs 6/70 p = 0.151	11/70 vs 9/70 p = 0.405*

* Includes adenomas and adenocarcinomas

For both sexes the level of statistical significance has not reached the borderline range of $p = 0.10 > p > 0.05$ for a one sided test. For the low dose males, a decrease in tumor incidence of approximately the same level of statistical significance as the increase at the high dose (i.e., $p = 0.145 \sim 0.151$) is observed. Because the data are inconsistent and because the incidences of lung tumors in both sexes fall within or below the range for other mice strains, including the CD-1 mouse (also a Swiss strain derived mouse, as is the Alderly Park mouse), Toxicology Branch is not certain that the incidence of lung neoplasia (see Table 2, p. 25) in either sex, in this study, is directly related to ingestion of Permethrin. However,

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since lung adenomas were also present in the FMC Mouse II and B-W Mouse studies, Toxicology Branch is cautious about disregarding the effect. There were no notable patterns of other (including rare, unusual, or malignant) neoplasms which occurred in treated or in the control animals.

As is the case in the majority of oncogenic studies, the time from initiation of exposure to tumor development can not be precisely determined in this study. However, the majority of the lung neoplasms were found at death or sacrifice of the animals late in the 98-week study interval. The mean time, in weeks, from initiation of the study to tumor discovery for control and each treated group is: males - 84.8, 91.5, 92.6, and 85.3; females - 81.3, 81.2, 86.7, and 87.3. This data does not suggest shortening of the latency period.

<u>FMC Mouse II Study</u> - Bio/Dynamics Inc.
Project No. 76-1695
October 9, 1979

Doses of 0 (control), 20, 500, and 2000 ppm of Permethrin were administered in the diet to groups of 75 male Charles River CD-1 strain mice and doses of 0 (control), 20, 2500 and 5000 ppm to groups of 75 female mice for 104 weeks. Initial concerns regarding abdominal distention and amyloidosis in animals of this study have been dismissed as having no significant influence on the interpretation of results. Relevant non-oncogenic effects observed during the study were increased mortality in males at 2000 ppm, increased liver weights in females at 2500 and 5000 ppm, and increased lung weights in females at 5000 ppm. Histopathologically, "focal areas of alveolar cell proliferation" (increased numbers of lung cells) was observed with dose-related incidence in Permethrin treated females . The incidence of this lesion is presented for both sexes in Table III.

Table III

FMC Mouse II Study

Mice Exhibiting Multifocal Alveolar Cell Proliferation

Males No.	Males %	Dosage Group	Females No.	Females %
1/75	1.3	I	3/75	4.0
7/75	9.3	II	5/76	6.6
5/74	6.8	III	11/75	14.7
1/75	1.3	IV	13/75	17.3

Multifocal hepatocytomegaly (increased liver cell volume) was observed with increased frequency in both sexes at the high dose levels and to a lesser extent in the other treated groups. Necrosis of the liver did not follow a dose-related pattern.

Table IV

FMC Mouse II Study

Livers Exhibiting Multifocal Hepatocytomegaly or Necrosis at Time of Sacrifice (S) or Death (D)

Males*						Dosage Group	Females*					
Cytomeg.			Necro.				Cytomeg.			Necro.		
S	D	Tot.	S	D	Tot.		S	D	Tot.	S	D	Tot.
0	1	1/75	0	13	13/75	I	0	0	0/74	2	6	8/74
2	4	6/75	2	6	8/75	II	1	2	3/76	3	8	11/76
3	4	7/75	2	5	7/75	III	3	3	6/76	1	6	7/76
3	11	14/75	1	10	11/75	IV	3	6	9/75	2	6	8/75

* Denominators are tissues examined.

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The mechanistic explanation of the hepatocytomegaly in this study is not obvious. It might be related to the enzyme induction observed or strongly suggested in all three mouse studies as discussed on p. 19 of this Summary, or it might be a precursor of necrosis (as suggested by the data in Table IV) and therefore a manifestation of Permethrin toxicity.

With regard to oncogenic effects, the initial pathology report (submitted with the study and dated February 7, 1980) indicated an increased incidence of bronchioalveolar adenomas in female mice. A second reading of the same lung slides (performed under contract to EPA; report dated February 23, 1981) also reported an increased incidence of alveolar cell neoplasms in female mice. Data from the second report is presented in Table V.

Table V

FMC Mouse II Study

Total Mice with Bronchioalveolar Neoplasms (Adenoma and/or Carcinoma)

Males		Dosage Group	Females	
No.	%		No.	%
23/75	30.7	I	15/75	20.0
20/75	26.7	II	24/76	31.6
28/74	37.8	III	35/75	46.7
21/75	28.0	IV	44/75	58.7

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The second pathology report also presented separate incidences for pulmonary adenomas and carcinomas. These are presented in Table VI.

Table VI

FMC Mouse II Study

Mice with Bronchioalveolar Adenoma (BA) or Carcinoma (BC)

Males				Dosage Group	Females			
BA		BC			BA		BC	
No.	%	No.	%		No.	%	No.	%
16/75	21.3	7/75	9.3	I	10/75	13.3	6/75	8.0
17/75	22.7	5/75	6.7	II	18/76	23.7	7/76	9.2
20/74	27.0	13/74	17.7	III	26/75	34.7	11/75	14.7
17/75	22.7	4/75	5.3	IV	37/75	49.3	15/75	20.0

N.B. [Caution must be exercised in comparing numerators in Tables V and VI. Because some mice bore both BA and BC the numerators in these Tables are not addable.]

The incidence of pulmonary neoplasms in male mice, Tables V and VI, does not appear to be related to treatment with Permethrin. The numbers of male mice with alveolar cell adenomas, with alveolar cell carcinomas, or with adenomas and/or carcinomas demonstrate no dose-dependence or suggestion that Permethrin treated males had a higher incidence of neoplasms that did the male controls. The slightly higher incidences observed in Group III are considered by TB to be within the limits of normal biological variation. (See Table 2, p. 25 for incidence of pulmonary tumor variability in CD-1 male mice, the mouse used in this study).

The denominators, presented below, for female mice are slightly modified from those given in the EPL Pathology Report, 2/23/81, and given in Table VI. Those mice for which the individual pathology sheets stated that autolysis had occurred, and no further diagnosis was made, were deleted.

The number of female mice with alveolar cell adenomas (10/74, 18/72, 26/74 and 37/75), with alveolar cell carcinomas (6/74, 7/72, 11/74 and 15/75) and with adenomas and/or carcinomas (15/74, 24/72, 35/74 and 44/75) demonstrate a significant dose-response. Statistical analyses of the incidence data for lung neoplasms in female mice revealed the following:

(1) Alveolar cell carcinomas (alone), when analyzed by Peto's Prevalence Method, demonstrated a significant positive time-adjusted dose-response trend with a one sided p value of 0.006. Pair-wise comparisons using chi-square, with Yates correction, gave a one sided p value of 0.15 and 0.032 for control vs 2500 ppm and control vs 5000 ppm respectively.

(2) Alveolar cell neoplasms (adenomas and/or carcinomas), when analyzed by Peto's Prevelence Method, demonstrated a significant positive trend with a one sided p value of 8.49 x 10^{-8}. Pair-wise comparisons using chi-square, with Yates correction, also gave significant values of 0.0005 and < 0.00001 for control vs 2500 ppm and control vs 5000 ppm respectively.

As stated previously in this document, most oncogenicity studies do not allow evaluation of "time to tumor" with a high degree of certainty. This is true in this study. However, Table VII presents an analysis, by Peto's Prevalence Method, of the female lung tumor/dose-response trends using the incidences given above.

Table VII

FMC Mouse II Study

Lung Tumor/Dose-Response Trends* for Female Mice

Study Interval (Months)	Alveolar Cell Carcinomas (alone) (p value)	Alveolar Cell Neoplasms - (Adenomas and/or Carcinomas) (p value)
1-12	0	0.59
13-18	0.3	0.33
19-21	0.4	0.003
22-24	0.002	0.006
1-24**	0.01	8.48×10^{-4}
24***	0.14	3.45×10^{-6}
Total Study****	0.006	8.49×10^{-8}

*   Peto's Prevalence Method (one sided significant test)
**   for entire in-life phase of study
*** for animals at terminal sacrifice only
**** for all animals in entire study

For alveolar cell carcinomas (alone), there was a significant dose-related increase in tumors during the final 3 months of the study which largely accounted for the significant increases also observed for the entire life phase of the study (months 1-24) and for the total study (all animals). For alveolar cell neoplasms (adenoma and/or carcinoma), there was a significant dose-related increase in tumors during the final 6 months of the study and significant increases for the entire life phase of the study, for the terminal sacrifice animals and for the

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total animals in the study. Historical control data for the specific mouse used in this study (CD-1) indicate that these tumor types regularly appear late in the lifespan, at a mean of 22.3 and 22.5 months of age for males and females respectively (1). In view of this, the total data base does not suggest a decrease in latency.

The above data is interpreted by TB as clearly indicating that administration of dosage levels of 2500 ppm and 5000 ppm of Permethrin to female mice in this study resulted in a significant dose-related increased incidence of alveolar cell neoplasms (adenoma and/or carcinoma). For alveolar cell carcinomas (alone), the data is somewhat less convincing at the dosage level of 2500 ppm, but there is nevertheless clear evidence of a significant dose-related increase in alveolar cell carcinomas, particularly at 5000 ppm. Permethrin apparently enhanced the normally expected spontaneous lung tumor incidences in the females, only, in this study.

The incidences of male and female mice with liver neoplasms, provided by the second pathology report, are presented in Table VIII.

Table VIII

FMC Mouse II Study

Total Mice with Liver Neoplasms

Males		Dosage Group	Females	
No.	%		No.	%
22/73	30.1	I	6/74	8.1
29/73	39.7	II	7/76	9.2
34/70	48.6	III	25/76	32.9
25/69	36.2	IV	30/75	40.0

The second pathology report also provided separate incidences for hepatomas and hepatocellular carcinomas. These are presented in Table IX.

Table IX

FMC Mouse II Study

Mice with Hepatoma (H) or Hepatocellular Carcinoma (HC)

Males				Dosage Group	Females			
H		HC			H		HC	
No.	%	No.	%		No.	%	No.	%
8/75	10.7	16/75	21.3	I	3/74	4.1	4/74	5.4
19/75	25.3	12/75	16.0	II	4/76	5.3	3/76	3.9
17/75	22.7	19/75	25.3	III	23/76	30.3	3/76	3.9
19/75	25.3	8/75	10.7	IV	29/75	38.7	2/75	2.7

N.B. [Caution must be exercised in comparing numerators in Tables VIII and IX. Because some mice bore both H and HC, the numerators in these Tables are not addable.]

The test for homogeneity of the distribution of liver neoplasms in males is marginally significant at $p = 0.0740$ (one sided test). In females, there was a statistically significant time adjusted dose-related trend (Peto's Prevalence Method) with $p \sim 5.5 \times 10^{-10}$ for liver adenoma and/or carcinoma. As can be seen readily in Table IX, the increase in hepatomas, and not hepatocellular carcinomas, accounted for the increased incidence in females. The incidence of hepatocellular carcinoma was not dose-related. There was no indication of a decreased latency period for liver tumors.

No notable patterns of other (including rare, unusual or malignant) neoplasms were present in this study.

A joint FDA-EPA audit of this study conducted in late 1980 at Bio/Dynamics and FMC facilities did not reveal any inadequacies in the conduct or reporting of this study serious enough to compromise the usefulness of these study results for oncogenic evaluation. However, the audit concluded that this study was not useful for assessment of chronic toxicity for a variety of reasons.

B-W Mouse Study - The Wellcome Foundation
Lab. No. HEFG 80-29
Received by EPA on December 17, 1980

Permethrin was administered in the diet to male and female CFLP strain mice for 92 weeks at dosage levels of 0 (control), 10, 50 and 250 mg/kg/day. One hundred mice/sex were used for the control groups and 75 mice/sex for each of the test groups. Relevant non-oncogenic effects observed during the study were slightly decreased mortality in females at 50 and 250 mg/kg/day, increased liver weights in males and increased kidney weights in females at 250 mg/kg/day. Histopathologically, an increased incidence of cuboidal/columnar metaplasia of the alveolar epithelium was observed in the lungs of male and female mice at the high dosage level (250 mg/kg/day).

Table X

B-W Mouse Study

Mice with Cuboidal/Columnar Metaplasia of Alveolar Epithelium*

Males		Dosage Group	Females	
No.	%	mg/kg/day	No.	%
0/99	0.0	0	0/96	0.0
1/75	1.3	10	0/71	0.0
1/73	1.4	50	1/74	1.4
3/74	4.1	250	5/74	6.8

* Based on total tissues examined.

Although controversial, this lesion is considered by some pathologists to be a precursor of lung neoplasms in mice.

The data in Table XI indicates a dose-related trend in females, but not in males, for adenomatous tumors in the lungs.

Table XI

B-W Mouse Study

Mice with One or More Adenomatous Tumors in the Lungs*

Males		Dosage Group	Females	
No.	%	mg/kg/day	No.	%
26/99	26.3	0	3/96	3.1
14/75	18.7	10	5/71	7.0
17/73	23.3	50	7/74	9.5
16/74	21.6	250	15/74	20.3

* Based on total tissues examined.

The registrant (B-W) has submitted statistical data with the report of this study showing that by adjusting for time of diagnosis, Peto's Prevalence Method indicates a statistically significant ($p < 0.01$) relationship between dose of Permethrin and lung tumors in females. When these data are evaluated by Armitage's method for linear trend, the level of statistical significance for the dose-response trend is $p = 0.02$. In addition, comparison of the number of tumors in the high dose group (15/74) to the number in the controls (3/96) shows statistical significance ($p = 0.0003$) by Fisher's Exact Test. Further, with respect to malignant lung tumors in these females, 1/74 animals in the mid dosage level group and 2/74 animals in the high dosage level group were diagnosed as having adenocarcinomas whereas this diagnosis was not made for any lung tumors in the control or low dosage level animals. The incidence of malignant lung tumors in males in this study was 2/99, 1/75, 2/73 and 1/74 for the control group and each of the treated groups respectively. The occurrence of three adenocarcinomas in the lungs of 219 Permethrin-treated females in this study does not indicate, in the opinion of TB, an increase in malignancy. Toxicology Branch considers the increased incidence of lung tumors in female mice observed in this study, together with the other supportive evidence observed in this study, to be highly suggestive of a possible oncogenic effect in lungs of females -- particularly when considered in relation to the results of the other two oncogenic studies in mice.

No notable patterns were observed for other neoplasms, including rare, unusual or malignant neoplasms, in any group. There were no indications of a decreased latency period for any kind of neoplasia.

ICI Rat Study - Central Toxicology Laboratory, ICI
Report No. CTL/P/357
Received by EPA on January 29, 1978

Doses of 0 (control), 500, 1000, and 2500 ppm of Permethrin were administered in the diet to 60 male and 60 female Wistar strain rats/group for 104 weeks. Eleven or twelve rats of each sex in each dosage group were sacrificed at 52 weeks and the remainder at 104 weeks. Relevant non-oncogenic effects observed during the study were increased mortality in males and decreased mortality in females at 2500 ppm, increased liver weights in males and females at 2500 and 1000 ppm and in males only at 500 ppm, increased liver enzyme (aminopyrine-N-demethylase) activity in males and females at 2500 and 1000 ppm, and hepatocyte vacuolization or hypertrophy in males and females at 2500 and 1000 ppm. Additional effects observed were increased kidney weights in males at all treatment levels, and increased pituitary weights in males at 2500 and 1000 ppm. Body tremors were also observed in males and females during the first 3 weeks of the study at 2500 ppm.

No tumors considered by Toxicology Branch to be related to or attributable to the ingestion of Permethrin were observed in this study.

FMC Rat Study - Bio/Dynamics Inc.
Project No. 74R-1022
November, 1977

Permethrin was administered in the diet to 60 male and 60 female Long-Evans strain rats per group for 104 weeks at dosage levels of 0 (control), 20, 100, and 500 ppm. Ten males and 8 females from the 100 ppm group were sacrificed at 52 weeks. The remaining animals were sacrificed at 104 weeks. Relevant non-oncogenic effects included increased liver weights for males at 100 and 500 ppm.

As mentioned earlier, this study has been judged to be of very limited usefulness with regard to evaluation of lung tumors because of serious flaws in histological methodology.

An increased incidence of adenomas and adenocarcinomas was initially reported in the lungs of male rats in this study. The reading of the original set of lung slides reported 1/59, 3/57, 6/57 and 5/56 lung neoplasms for control, low, mid and high dosage level males respectively. This increased incidence was not statistically significant ($p > 0.05$). These same lung slides were later reread by a second pathologist who reported 1/60, 3/57, 8/60 and 6/60 lung neoplasms for the respective groups. Based on the readings of the second pathologist, the increase in lung neoplasms was statistically significant ($p < 0.05$) for the mid and high dosage level males relative to the male control group. In a subsequent effort to perform a more critical and detailed evaluation of lung tumors in male rats, all available lung tissues from the male animals were step-sectioned at 250 micron intervals to exhaustion of tissue and read by the original pathologist who then reported revised figures of 8/60, 6/57, 10/60 and 10/60 for the respective groups (based on original and step-sectioned slides). This finding was not statistically significant ($p > 0.20$). Based on information supplied by FMC and on a joint FDA-EPA audit of the laboratories and personnel involved in the histological processing of the lung slides, Toxicology Branch has determined that inconsistencies in the technical methodologies used in the original sectioning and processing and also later in the step-sectioning and processing of these lung tissues introduced serious bias into all these results--largely due to inconsistent embedding techniques which resulted in unequal amounts of lung tissue being examined in the control and test groups. Readings of the original lung slides were biased in favor of finding relatively more tumors in the lungs of test animals whereas readings of the step-sectioned slides were biased in favor of finding relatively more tumors in the lungs of control animals.

In an effort to resolve the dilemma of which figures to use, Toxicology Branch has made additional theoretical calculations of lung tumor incidence based on equal amounts of lung tissue (adjusted) from all control and test groups. These "area adjusted" tumor incidences were calculated to be 1/60, 2/57, 5/60 and 4/60 for the original (non-step-sectioned slides) and 8/60, 8/57, 15/60 and 14/60 for all slides (original and step-sectioned slides). The statistical significance of these "area adjusted" findings was borderline (p approximately 0.10).

The incidence of alveologenic tumors among the females was 1/60, 1/60, 1/59, and 2/59 for the control, low, mid and high dosage level groups respectively. All alveologenic tumors in males and all but two of those found in the females were discovered at terminal sacrifice, suggesting that this type of tumor is late appearing. This type of tumor has a low incidence in historical control rats.

Evidence suggests the possibility of an oncogenic effect occurring in the lungs of treated male rats in this study. Sufficient uncertainty regarding the validity of the incidence figures, however, precludes making a scientifically supportable evaluation of the results.

Regarding other neoplasms, no dose related increases were noted. No rare, unusual or malignant neoplasms were found which could be related to the ingestion of Permethrin. No decrease in latency period was noted.

B-W Rat Study - The Wellcome Foundation
Lab. No. HEFG 80-33
July 2, 1980

Permethrin was administered in the diet to 60 male and 60 female Wistar strain rats per group for 104 weeks at dosage levels of 0 (control), 10, 50 and 250 mg/kg/day. Relevant non-oncogenic effects observed during the study were increased mortality in males at 250 mg/kg/day, occasional body tremors in males and females at 250 mg/kg/day, increased liver weights in males at 250 mg/kg/day, hepatocyte hypertrophy in males and females at 250 mg/kg/day and focal disturbances in the growth pattern of thyroid follicular cells in males and females at 250 mg/kg/day. The microscopic liver and thyroid changes were also observed in males and females at 50 mg/kg/day.

With respect to tumors (including rare, unusual or malignant neoplasms), none of the tumor types observed in this study were considered by Toxicology Branch to be related to or attributable to the ingestion of Permethrin. There was no suggestion of a decrease in latency period in this study.

III. Non-Oncogenic NOEL for Mouse Studies:

Relevant non-oncogenic effects observed in the 3 long-term mouse studies are tabulated below.

Study	Dosage Level of Permethrin (ppm)		Non-Oncogenic Effects
ICI Mouse	0	-	None (M&F)
	250	-	None (M&F)
	1000	-	Minimal changes in liver enzyme activity, liver weights and liver histopathology (eosinophilia of hepatocytes) (M&F)
	2500	-	Increased mortality (M&F), Increased liver enzyme activity (M&F), Increased liver weights (M&F), Eosinophilia of hepatocytes (M&F)
FMC Mouse II	0	-	Multifocal alveolar cell proliferation (M&F), Multifocal hepatocytomegaly (M)
	20 (M&F)	-	Multifocal alveolar cell proliferation, Multifocal hepatocytomegaly
	500 (M)	-	Multifocal alveolar cell proliferation, Multifocal hepatocytomegaly
	2000 (M)	-	Multifocal alveolar cell proliferation, Multifocal hepatocytomegaly, Increased mortality.
	2500 (F)	-	Multifocal alveolar cell proliferation, Multifocal hepatocytomegaly, Increased liver weights, Hepatocyte pigmentation.
	5000 (F)	-	Increased liver weights, Multifocal alveolar cell proliferation, Multifocal hepatocytomegaly, Hepatocyte pigmentation, Increased lung weights.

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B-W Mouse	0	-	None (M&F)
	10*	-	None (M&F)
	50*	-	None (M&F)
	250*	-	Slightly increased liver weights (M), Slightly increased kidney weights (F), Cuboidal/columnar metaplasia of alveolar epithelium in lungs (M&F)

* mg/kg/day (M) = Male (F) = Female

A consistent finding in all 3 mouse studies at high dosage levels was liver changes known to be associated with induction of the liver microsomal enzyme system. These changes included slightly increased liver weights and increased enzyme (aminopyrine-N-demethylase) activity. This induction phenomenon is a well-known and frequently occurring response to the administration of many exogenous compounds to experimental animals and man. It is widely accepted among knowledgeable scientists as being a normal and natural adaptive response of the organism to the presence of foreign chemicals and is not considered to be an adverse or toxicological effect of concern. Therefore, Toxicology Branch notes the presence of this phenomenon in mice treated with Permethrin, but will not use it to determine a NOEL. Of potentially more concern are other histopathological effects observed in liver cells that are not ordinarily associated with microsomal induction and could possibly be related to an adverse effect of Permethrin on the liver. These effects include liver weight increases, multifocal hepatocytomegaly, hepatocyte pigmentation and possibly eosinophilia of hepatocytes. These and other toxicological manifestations were used to determine a non-oncogenic NOEL for the mouse studies.

Examination of the non-oncogenic effects in mice reveals that liver effects were present in the ICI Mouse Study at 1000 ppm (150 mg/kg/day) and higher, and in the B-W Mouse Study at 250 mg/kg/day. It will also reveal that liver effects were not present in these studies at levels of 250 ppm (37.5 mg/kg/day) and 50 mg/kg/day. The unusual pattern (see Table IV, p. 5) of distribution of multifocal hepatocytomegaly and necrosis of the liver cells in the FMC Mouse II study (i.e. in control and treated groups) is very difficult to interpret. These effects could be attributable to the general animal health problems observed in this study or, in the case of hepatocytomegaly, to the ingestion of Permethrin. The Toxicology Branch takes the position that the hepatocytomegaly observed in the 2000, 2500 and 5000 ppm (300, 375 and 750 mg/kg/day) level mice is treatment related. The weight of evidence favors the 50 mg/kg/day level in the B-W Mouse Study as an appropriate mouse non-oncogenic NOEL.

IV. Non-Oncogenic NOEL for Rat Studies:

Relevant non-oncogenic effects observed in the 3 long-term rat studies are tabulated below.

Study	Dosage Level of Permethrin (ppm)		Non-Oncogenic Effects
ICI Rat	0	-	None
	500	-	Increased liver weights (M), Increased kidney weights (M)
	1000	-	Increased liver enzyme activity (M&F) Increased liver weights (M&F), Hepatocyte vacuolation (M&F), Hepatocyte hypertrophy (M&F), Increased kidney weights (M), Increased pituitary weights (M)
	2500	-	Increased mortality (M), Increased liver enzyme activity (M&F), Increased liver weights (M&F), Hepatocyte vacuolation (M&F), Hepatocyte hypertrophy (M&F), Increased kidney weights (M), Increased pituitary weights (M), Body tremors (1st 3 weeks) (M&F)
FMC Rat	0	-	None
	20	-	None
	100	-	Slightly increased liver weights (M)
	500	-	Increased liver weights (M)
B-W Rat	0	-	None
	10*	-	None
	50*	-	Hepatocyte hypertrophy (M&F) Changes in thyroid cells (M&F)
	250*	-	Increased mortality (M), Increased liver weights (M), Hepatocyte hypertrophy (M&F), Changes in thyroid cells (M&F), Occasional body tremors (M&F)

* mg/kg/day (M) = Male (F) = Female

As with mice, a consistent finding in all 3 rat studies was liver changes known to be associated with induction of the microsomal enzyme system. This induction phenomenon, for reasons already presented, was again not considered by Toxicology Branch to be an adverse or toxicological effect of concern. The other toxic effects listed above were used to determine a non-oncogenic NOEL for the rat studies.

The NOEL for each study was judged to be: FMC Rat Study, 100 ppm or 5 mg/kg/day; ICI Rat Study, < 500 ppm or < 25 mg/kg/day; and the B-W Rat Study, 10 mg/kg/day. Based on the weight of evidence in all three rat studies, the non-oncogenic NOEL for rats is judged to be 5 mg/kg/day.

V. Overall Assessment of the Oncogenic Potential in Experimental Animals:

According to the International Agency for Research on Cancer (IARC), "the widely accepted meaning of the term 'chemical carcinogenesis' --- is the induction by chemicals of neoplasms that are not usually observed, the earlier induction by chemicals of neoplasms that are usually observed, and/or the induction by chemicals of more neoplasms than are usually found" (2). The Toxicology Branch considers this definition and the following seven criteria to evaluate oncogenicity in experimental animals when multiple oncogenic studies are available on a single chemical:

1. Oncogenicity in different a) species, b) strains, c) sexes and d) organs

Three strains of mice were used in the long-term mouse studies: a) ICI, Alderly Park, (SPF Swiss derived); b) FMC II, Charles River CD-1, (Swiss derived); and c) B-W, CFLP, (Swiss derived). In the ICI study, a slightly increased incidence of lung adenomas was observed in male mice and lung adenomas and/or adenocarcinomas in female mice at the highest level fed (see Table II, p. 3). It is not at all certain that the incidence of lung neoplasia in either sex, in this study, is directly related to treatment. Females in the FMC II study exhibited an increased incidence of lung and liver tumors at the two highest levels fed (see Table V, p. 6 and Table VIII, p. 10). In the B-W study, female mice exhibited an increased incidence of lung tumors at the highest level fed (see Table XI, p. 13). This increase in lung tumors is considered to be suggestive of a possible oncogenic effect in the lungs of female mice in this study. Only females of the Charles River CD-1 strain (FMC II study) showed clear evidence of lung and liver oncogenicity apparently due to Permethrin exposure.

Among the three long-term mouse studies, clear evidence of Permethrin oncogenicity was observed in the lungs and livers of only one sex in one strain of mouse. Suggestive evidence of lung tumors in a second strain, females only, at the highest level fed was also observed.

Of the three long-term rat studies, the ICI (Wistar strain) and the B-W (Wistar strain) studies revealed no tumors, in either sex, which were considered to be related to or attributable to the ingestion of Permethrin. The flawed FMC (Long-Evans strain) study produced evidence suggestive of an oncogenic effect in the lungs of males only.

2. Presence of rare neoplasms and number of different types of neoplasms in one or more species

Lung and liver tumors were the predominant neoplasms of potential concern in the long-term mouse studies. In mice, lung and liver tumors are not rare neoplasms (see Table 2, p. 25). Both tumor types occur spontaneously in control mice and have highly variable incidence rates (occasionally quite high) from study to study. With the exception of a marginal and questionable effect in males in the ICI study, increased incidences of lung tumors were observed only in females. An increase in the number of hepatomas was confined to females in the FMC Mouse II study. An increased incidence for this tumor type did not occur in either sex of the ICI or B-W studies. Other types of tumors observed in either sex in the three mouse studies were not attributed to ingestion of Permethrin.

No tumors considered to be related to ingestion of Permethrin were observed in the ICI or B-W Rat studies. Evidence in the flawed FMC Rat study suggests the possibility of an oncogenic effect in the lungs of male rats. The uncertainty of incidence figures in this study, however, precludes making a scientifically supportable evaluation of this observation (see pp. 15-16 for detailed discussion). Lung adenomas are not a common tumor type in this species.

Rare or unusual tumors which might be attributable to treatment with Permethrin were not observed in any of the five adequately executed mouse or rat long-term studies.

3. Increased incidence of malignant neoplasms

The number of malignant lung tumors at termination of the ICI Mouse study was small. For males, the incidence was 0/70 for all groups. For females, the incidence was 0/70, 1/70, 1/70 and 1/70 for the control and the three treated groups respectively (see Table II, p. 3). This was also true at termination of the B-W Mouse study. The incidence for males was 2/99, 1/75, 2/73, 1/74 and for females 0/96, 0/71, 1/74, 2/74 for the control group and each of the treated groups respectively (see p. 13). At termination of the FMC Mouse II Study, an increased incidence of alveolar cell neoplasms (adenomas/carcinomas) was observed in females only. The number of adenomas alone was 10/74, 18/72, 26/74, and 37/75 for the female control and treated groups respectively. The number of lung carcinomas in these same groups was 6/74, 7/72, 11/74, 15/75 respectively (see p. 8). Only the high level females exhibited a significantly increased lung carcinoma incidence relative to the control females (chi-square test with Yates correction). For males, the incidence of lung carcinoma was 7/75, 5/75, 13/74 and 4/75 (see Table VI, p. 7). In this same study, the incidence, at termination, of hepatocellular carcinoma among females was 4/74, 3/76, 3/76, 2/75 for the control and each of the treated groups respectively and among males was 16/75, 12/75, 19/75 and 8/75 respectively (see Table IX, p. 11).

With respect to tumors of any kind, including malignant tumors, in the ICI and B-W Rat studies, none were considered by Toxicology Branch to be related to or attributable to the ingestion of Permethrin. No scientifically supportable evaluation of the lung tumor incidence observed in the FMC Rat study is possible. No evidence of increased incidence of malignant tumors was observed in any of the rat studies.

Histopathological evidence for increased malignant lung tumors was observed in only one sex in one of six mouse and rat studies. Evidence for increased malignant liver tumors was not observed.

4. Decrease in latency (time to tumor discovery)

As is the case in the majority of oncogenicity studies, the time from initiation of exposure to tumor development can not be precisely determined. However, the majority of the lung neoplasms in the ICI Mouse study were found at death or sacrifice of the animals late in the study as were those in the B-W Mouse study (see p. 4 and p. 14). In the FMC Mouse II study, although a sensitive statistical analysis of the female lung tumor/dose response trends suggested a possible decrease in latency, this was not confirmed when the same data was compared to the latency time for lung tumors in historical control mice. Considering all the evidence, TB concluded this data did not indicate a decrease in latency for the lung tumors in the female mice in this study (see pp. 9-10 for discussion). The liver tumors in this same female group were also discovered late in the study (see p. 11). Decreased latency was not observed in any of the three long-term mouse studies.

No decreased latency for lung tumors was observed in the ICI or B-W long-term rat studies. The problem with lung histopathology data in the FMC Rat study precluded any evaluation of latency period for lung neoplasms. No decrease in latency period was noted for any other neoplasms in this study.

A decreased latency period was not observed in any of the long-term mouse or rat studies.

5. Dose-response relationships

In the ICI Mouse study, no substantive evidence was developed which indicated a dose-response relationship for lung adenomas in either sex. A dose-related trend in lung adenomas was observed, for females only, in the B-W Mouse study. The FMC II Mouse study indicated a dose-related response for both lung and liver neoplasms for females only. No dose-response relationships for tumors were observed in either sex in any of the long-term rat studies.

6. Mutagenicity tests

A battery of mutagenicity tests has been performed on Permethrin to detect gene mutations, chromosomal aberrations and primary DNA damage. These tests included studies on *S. typhimurium* and *E. coli* (with and without activation), mouse lymphoma, dominant lethal, rat cytogenetics, mitotic recombination in yeast, DNA repair in *E. coli* and *B. subtilis* and unscheduled DNA synthesis in human fibroblasts. In none of these studies has Permethrin shown a mutagenic potential.

The mechanism of tumor induction by Permethrin apparently does not operate by biological mechanisms which directly involve the genetic integrity of the cell.

7. Spontaneous tumor incidence in untreated animals

Limited historical control tumor incidence data for the particular strains of mice and rats in the six long-term studies is available to the Toxicology Branch. Homburger et. al. (1) have published such data for the CD®-1 HaM/ICR Mouse, a Swiss derived strain, used in the FMC Mouse II Study. Table 2 presents the spontaneous tumor incidence for this strain of mouse.

TABLE 2.—*Incidence of tumors during life-span in groups of untreated CD®-1 HaM/ICR mice* **

Group	Final No.		Percent with tumors		Average No. of tumors/mouse		Percent with multiple tumors		Percent lymphoma-leukemia		Percent lung tumors		Percent hepatomas		Percent vascular tumors		Percent other tumors	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
I	18	20	67	85	1.2	1.2	33	30	33	30	28	30	17	0	17	0	39	36
II	14	15	57	73	0.6	0.8	7	7	14	7	29	60	7	0	0	0	14	13
III	16	13	56	69	0.7	1.0	13	23	13	15	13	23	6	0	13	15	25	46
IV	14	15	50	67	0.7	0.9	14	20	14	13	28	33	0	7	14	13	14	20
V	19	17	21	65	0.2	0.8	0	17	5	35	5	24	5	0	0	6	5	17
VI	18	22	72	82	0.9	1.1	16	23	22	50	44	23	6	0	6	18	16	18
Total	99	102																
Average			54	75	0.7	1.0	14	21	17	31	24	31	7	1	5	9	19	25

* Initial number of mice in all groups was 28 ** From (1)

In the FMC Mouse II Study, the incidence of lung tumors was 31, 27, 38 and 28% for males and 20, 32, 47 and 59% for females (see Table V, p. 6). The upper incidences are encompassed by the range for males (5-44%) and females (23-60%) presented in Table 2 for this strain of mouse. The incidence of these tumors in the Alderly Park mouse, also a Swiss derived strain, used in the ICI Study was 16, 9, 19 and 24% for males and 16, 11, 14 and 21% for females (see Table II, p. 3). Similarly, the incidence of these tumors in the CFLP mouse, also a Swiss derived strain, used in the B-W Study was 26, 19, 23 and 22% for males and 3, 7, 10 and 20% for females (see Table XI, p. 13). These upper incidences also are encompassed by the ranges for the respective sexes in Table 2.

Data submitted by one registrant presented the historical control incidence of lung tumors in female mice of the CFLP (Swiss derived) strain used in the B-W study. The data for 807 female control mice from nine studies was abstracted from proprietory data unrelated to Permethrin. This fact precluded proper validation and analysis. Nevertheless, the female control incidence for lung tumors ranged from 7.5 to 30.0% with a mean of 165/807 = 20.4%. The incidence in the B-W Permethrin study, as previously presented, was 3, 7, 10 and 20% for the female control, low, mid and high level treated groups respectively. These incidences clearly fall within and below the historical range for this type of tumor in CFLP mice.

Comparison of the observed lung tumor incidences in the three long-term mouse studies with the historical control incidences for either sex raises the possibility that these tumors may not be directly related to the ingestion of Permethrin but, rather, may be simply an expression of the variability of the spontaneous and naturally occurring incidence of this lesion in mice. This is particularly true for males for which no tumor/dose relationships were found. Toxicology Branch recognizes some merit in this point of view. However, the definite lung tumor/dose relationship found for the females in the FMC Mouse II Study and the lung tumor/dose related trend for the females in the B-W Mouse Study can not be ignored. Toxicology Branch considers the lung tumor incidences observed in females in these two studies to be supportive of one another.

In the FMC Mouse II Study, the increased incidence of liver tumors observed in males and females was due to an increase in hepatomas, and not to hepatocellular carcinomas. The incidence of hepatomas was 11, 25, 23 and 25% for males and 4, 5, 30 and 39% for females (see Table IX, p. 11). Comparison of these hepatoma incidences with the range for untreated mice of the same strain and sex (Table 2, p. 25) indicate that for males (0-17%), the FMC Study incidences tend to fall just slightly above the historical control range. For females (0-7%), however, the incidences of hepatomas in the mid and high dosage level groups clearly fall high above the historical control range.

The incidence of lung neoplasms in the flawed FMC Rat Study, in the absence of such observations in the ICI and B-W rat studies and specific historical control incidence data for the Long-Evans rat strain, is very difficult to interpret. Their presence, however, is noted.

Summary and evaluation of criteria data

1. In the 3 long-term mouse studies, frank evidence of carcinogenicity was observed in the lungs and liver of only one sex (female) of one strain (Charles River CD-1) of mice. There is suggestive evidence for increased trend for lung tumors in females of the B-W Mouse Study. The flawed FMC Rat (Long-Evans strain) study also produced evidence suggestive of an oncogenic effect in the lungs of males only.

2. Lung and liver neoplasms were the predominant neoplasms of concern in the mouse studies. In mice, lung and liver tumors are not rare neoplasms. In rats, suggestive evidence of increased incidence of lung adenomas was observed in male rats in a flawed long-term study. Lung adenomas are not a common tumor type in rats. There was no increased incidence of rare or unusual tumors in the mouse or the rat studies which was attributed to treatment with Permethrin.

3. An increase in malignant lung tumors was evident in only one sex in one of six long-term mouse and rat studies. No increase in malignant liver tumors was observed.

4. These studies were not designed to detect latency differences between groups. Clear evidence of a decreased latency period was not observed for any tumor type in any of the long-term mouse or rat studies.

5. Dose-related responses for lung and liver tumors were established for female mice in the FMC Mouse II Study and for lung tumors for female mice in the B-W Mouse Study.

6. In a battery of mutagenicity tests performed to detect gene mutations, chromosomal aberrations and primary DNA damage, no mutagenic potential was evident.

7. An examination of historical control data for untreated mice raises the possibility that lung tumors observed in these studies may be unrelated to treatment with Permethrin. Tumor/dose relationships in females in two studies, however, can not be ignored. The incidence of hepatomas in one sex of one strain of mouse lies above the historical control range. Historical control data for rats of the same strain is too limited to be of use in evaluation of the FMC Rat Study or the other two rat studies.

Evaluation of the weight of toxicological evidence leads the Toxicology Branch to conclude that, at dose levels above 250 mg/kg/day for a lifetime, Permethrin exhibits a low oncogenicity potential in female mice. While the evidence in the FMC Rat Study is flawed to the extent that it precludes making a scientifically supportable evaluation, it is marginally suggestive that Permethrin may also have a very low oncogenic potential in male rats. This potential is not supported by the other two long-term rat studies at dosage levels as high as 125 and 250 mg/kg/day.

The mechanism of induction of oncogenicity observed in rodents apparently does not operate by biological mechanisms which directly involve the genetic integrity of the cell.

VI. Assessment of the Oncogenic Potential in Humans:

Recently, several systems for ranking and classifying evidence from animal oncogenic studies have been developed or proposed. At present, only the International Agency for Research on Cancer (IARC) method has general acceptance. However, in evaluating the human oncogenicity potential for Permethrin, two additional systems were useful.

The IARC method classifies the evidence as either "sufficient" or "limited". Evidence which is "sufficient" requires the animal experiments to demonstrate "an increased incidence of malignant tumors: (i) in multiple species or strains, and/or (ii) in multiple experiments (routes and/or doses), and/or (iii) to an unusual degree (with regard to incidence, site, type, and/or precocity of onset)." (3). Data concerning dose-response relationships, mutagenicity, and chemical structure may provide additional evidence. "Limited" evidence, while not precisely defined by IARC, includes induction of "certain neoplasms, <u>including lung tumors and hepatomas in mice</u>, which have been considered of lesser significance than neoplasms occurring at other sites for the purpose of evaluating the carcinogenicity of chemicals", underlining added (4).

Using the IARC criteria, Permethrin animal data does not meet the requirements for the "sufficient" evidence category. Among six long-term mouse and rat studies, an increase in malignant lung tumors was not evident except in one sex (female) in the Charles River CD-1 mouse strain used in the FMC Mouse II Study. This tumor type and site is not rare or unusual for mice. Except for the FMC Mouse II Study and B-W Mouse Study, no definite dose-response relationships were established for tumor incidences. No evidence of mutagenic potential was observed in a battery of tests which included tests for DNA damage.

Using the IARC criteria and considering all available biological data, Toxicology Branch concludes that the evidence for Permethrin carcinogenicity must fall into the "limited" classification. The "limited" evidence strongly suggests that Permethrin is not a proven carcinogen in experimental animals but may exhibit a low oncogenic potential for female mice.

Weisburger and Williams (5) have proposed a mechanistic classification of oncogens which divides them into two general categories: a) genotoxic and b) epigenetic. The genotoxic category contains those oncogens which function as electrophilic reactants or otherwise affect DNA. The epigenetic category contains oncogenic substances for which no evidence of direct interaction with genetic material exists. The authors state:

> "This classification, if ultimately validated, has major implications for risk extrapolation to humans of data on experimental carcinogenesis. Genotoxic carcinogens, because of their effects on genetic material, pose a clear qualitative hazard to humans. These carcinogens are occasionally effective after a single exposure, act in a cumulative manner, and act together with other genotoxic carcinogens having the same organotropism. Thus, the level of human exposure acceptable for 'no risk' to ensue needs to be evaluated most stringently in the light of existing data and relevant mechanisms. Often, with powerful carcinogens, zero exposure is the goal.
>
> On the other hand, with some classes of epigenetic carcinogens, it is known that their carcinogenic effects occur only with high and sustained levels of exposure that lead to prolonged physiologic abnormalities, hormonal imbalances, or tissue injury. Consequently, the risk from exposure may be of a quantitative nature. This is almost certainly the case with estrogens, which are carcinogenic at high, chronic exposure levels in animal studies, or otherwise every individual would develop cancer. Thus, with epigenetic carcinogens, it may be possible to establish a 'safe' threshold of exposure, once their mechanism of action is elucidated." (6).

Because of the lack of knowledge concerning Permethrin's mechanism of action, the entire Weisburger and Williams classification system can not be fully utilized. However, the lack of positive evidence for mutagenic potential from a battery of tests, including DNA repair and unscheduled DNA synthesis, coupled with oncogenic evidence only at high dose levels for one sex in one mouse study among three studies, indicates that Permethrin falls into the epigenetic category. According to this system of classification, Permethrin falls into the group where the risk from exposure may be of a quantitative nature.

Squire (7) has also proposed a system for ranking animal carcinogens based on available data and the current state of knowledge. The system's "emphasis is on test animal data, since without further knowledge of mechanisms, this information is the most relevant to human risk. Whatever experimental data are to be included, however, the weight of scientific evidence should be considered in an appropriate system of carcinogen classification." (8). The system includes the following factors: a) number of different species affected; b) number of histogenetically different types of neoplasms in one or more species; c) spontaneous incidence in appropriate control groups of neoplasms induced in treated groups; d) dose-response relationships; e) malignancy of induced neoplasms; and f) genotoxicity, measured in an appropriate battery of tests. Weighted numerical values were assigned to subparts of these factors by the author. Using these values the author ranks carcinogens into five classes. Classes I and II contain substances presenting the greatest potential hazard and have the highest priority for regulation. According to the author, chemicals in Classes III to V may permit many regulatory options including no action, approvals for limited uses, appropriate labeling, or public education programs.

Using Squire's system of ranking potential carcinogens, Toxicology Branch has determined that Permethrin clearly falls into Class V--which indicates the lowest potential risk of carcinogenicity.

The weight of scientific biological evidence produced by six long-term mouse and rat studies plus the use of three oncogenicity ranking and classification systems lead the Toxicology Branch to conclude that Permethrin's potential for induction of oncogenicity in experimental animals is low and that the likelihood of oncogenic effects in humans is nonexistent or extremely low.

References

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